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Cross Transmission of 'Giardia'

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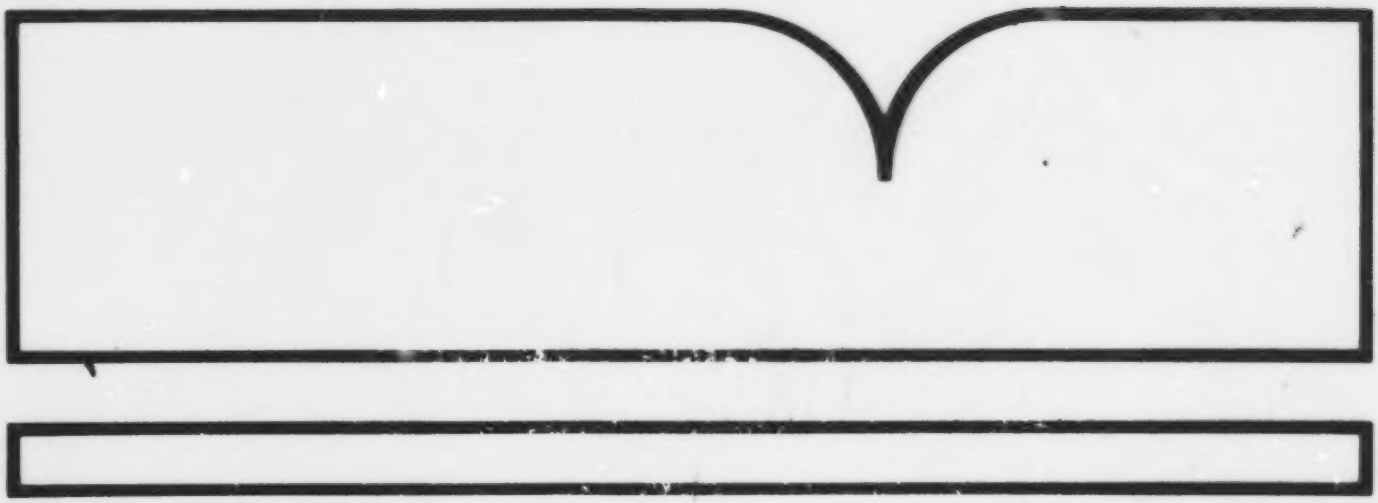

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CROSS TRANSMISSION OF GIARDIA

by

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16. ABSTRACT

Giardia cysts isolated from fecal samples obtained from humans (Homo sapiens), beaver (Castor canadensis), dogs (Canis familiaris), cats (Felis domesticus), bighorn X mouflon sheep (Ovis canadensis X O. musimon), guinea pig (Cavis porcellus), muskrat (Ondatra ziethica) and mule deer (Odocoileus hemionus) were given to a variety of experimental animals. Human source Giardia established infections in dogs, cats, beaver, rats (Rattus norvegicus), gerbils (Gerbillus gerbillus), guinea pig, raccoon (Procyon lotor), bighorn X mouflon sheep and pronghorn (Antilocapra americana). Giardia cysts from beaver successfully infected dogs. A dog was infected with Giardia from a bighorn X mouflon sheep which was infected with human source Giardia. Human source Giardia was used to infect cats and cysts from the cats were used to successfully infect dogs.

Evidence exists that once dogs are treated with metronidazole and then re-exposed to Giardia they become infected yet do not shed cysts. This most likely occurs in natural cases of giardiasis in dogs where the animal stops shedding cysts yet has a latent infection. Female dogs and cats may start shedding Giardia cysts 3-4 weeks after parturition.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of the environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory conducts a coordinated environmental health research program in inhalation toxicology, genetic toxicology, neurotoxicology, developmental and experimental biology and clinical studies using human volunteer subjects. These studies address problems in air pollution, water pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides and other chemical pollutants. The Laboratory participates in and provides data for the development and revision of criteria documents on pollutants for which national ambient air quality and water quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U. S. inhabitants.

This report describes the results of a survey for Giardia in domestic and wild animals and the subsequent inoculation of Giardia from positive animals into SPF dogs used as a surrogate for humans. Giardia cysts obtained from humans were tested in various animal species to determine their ability to act as carriers of this protozoan in the environment. A better understanding of the relationship of this organism within and between various animal species will aid in the epidemiological study of disease outbreaks and develop measures for water supply treatment where necessary.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

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ABSTRACT

Giardia cysts isolated from fecal samples obtained from humans (*Homo sapiens*), beaver (*Castor canadensis*), dogs (*Canis familiaris*), cats (*Felis domesticus*), bighorn X mouflon sheep (*Ovis canadensis* X *O. musimon*), guinea pig, (*Cavis porcellus*), muskrat (*Ondatra ziethica*) and mule deer (*Odocoileus hemionus*) were given to a variety of experimented animals. Human source *Giardia* established infections in dogs, cats, beaver, rats (*Rattus norvegicus*), gerbils (*Gerbillus gerbillus*), guinea pig, raccoon (*Procyon lotor*), bighorn X mouflon sheep and pronghorn (*Antilocapra americana*). *Giardia* cysts from beaver successfully infected dogs. A dog was infected with *Giardia* from a bighorn X mouflon sheep which was infected with human source *Giardia*. Human source *Giardia* was used to infect cats and cysts from the cats were used to successfully infect dogs.

Evidence exists that once dogs are treated with metronidazole (Flagyl®) and then re-exposed to *Giardia* they become infected yet do not shed cysts. This most likely occurs in natural cases of giardiasis in dogs where the animal stops shedding cysts yet has a latent infection. Female dogs and cats may start shedding *Giardia* cysts 3-4 weeks after parturition.

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SECTION 1

INTRODUCTION

Giardia (Protozoa: Hexamitidae) has been known as a parasite of humans since Leeuwenhoek found trophozoites of the protozoan in his own feces (1). A recent report has shown *Giardia lamblia* is the most common parasite in stool specimens submitted for examination in the United States with prevalences ranging from 2 to 20% and averaging 3.8% (3). Within Colorado, giardiasis exists in 2 forms: endemic and epidemic. Previous parasitological surveys have shown that *Giardia* is the most common parasite identified, with prevalence rates of 5% (4) and 5.6% (5) in 2 towns which had outbreaks of giardiasis. A third survey in Colorado showed 3% of the people examined were infected with *Giardia*. Infected people had watery diarrhea which lasted an average of 3.8 weeks. A correlation between seasonal distribution of cases and fecal contamination of mountain streams indicate drinking untreated water is an important cause of endemic giardiasis (6).

Numerous epidemics of giardiasis have occurred in Colorado with outbreaks in Aspen (4), Vail (7), Boulder (8), Estes Park (3) and various resort lodges (9, 10). The precise source of the *Giardia* in the above outbreaks is not known but *Giardia* cysts have been recovered from samples of public water supplies in Camas, Washington; Rome, New York; Estes Park, Colorado; and Berlin, New Hampshire (11). Cross connections between sewage and water lines were determined to be the cause of the 1965-66 Aspen, Colorado, outbreak (11), and the remainder were associated with incompletely treated surface water. Beaver (*Castor canadensis*) infected with *Giardia* were found below the water inlets for the Camas water system (12).

Sylvatic *Giardia* has been described in Colorado (13) with beaver (44 of 244 samples), cattle (6 of 58), domestic cat (1 of 4) and dogs (10 of 78) being positive for *Giardia*. Two of 34 coyotes (*Canis latrans*) from Northern New Mexico were also positive for *Giardia*.

Early parasitologists describing species of *Giardia* from the various hosts were prone to name species after the host in which they were found (e.g., *equi*, *canis*, *cati*, *caprae*, *bovis*, etc.) irrespective of morphologic similarities between *Giardia* in the different hosts. In the absence of cross transmission experiments to determine the validity of species, this probably was the safest approach. However, as early as 1952, Filice (17) could not find any morphologic difference between species of *Giardia* described from the laboratory rat and a number of wild rodents (14). In a review of the literature he discovered that most experimental cross-transmissions were unacceptable. This prompted Filice to propose 2 species, *G. muris* in the mouse, rat and hamster, and *G. duodenalis* in the rabbit, man, dog, cat and cattle as well as various rodents.

Although Filice's proposed speciation was based on morphology, the implication, that other animals could serve as reservoirs for man, was extremely important. While Filice apparently did not accept the success obtained by Hegner (18) and Armaghan (19) in infecting laboratory rats with Giardia from man, these early investigators were aware that the various Giardia might not be host specific.

Padchenko and Stolyarchuk (16) gave human-source Giardia to dogs in 1969 and reported establishing infections, the prepatent period ranging from 3 to 40 days. Unfortunately, the experiment was not well controlled. Not only is a 3 day prepatent period too short for Giardia, 40 days is too long; therefore, their results were unacceptable. In 1971 Hibler (unpublished) infected 6 dogs with human source Giardia, noting that the prepatent period was 6 to 9 days. While the dogs were carefully examined for evidence of Giardia infection for two weeks prior to experimental exposure, like Padchenko and Stolyarchak, the experiment was not adequately controlled. Nevertheless, the results strongly suggested that Filice's premise about speciation was probably correct.

In other cross-transmission studies, Grant and Woo (15 found that G. muris from laboratory mice, G. simoni from laboratory rats, and G. peromysci from deer mice were very host specific, while G. microti and G. mesocricetus were not host specific.

These cross-transmission studies were stimulated, to a certain extent, by the increasing number of unexplained epidemics of giardiasis in humans, all apparently waterborne, but not readily traceable to human contamination of the water supplies. They pointed to another possible source of contamination, wild or domestic mammals. This heuristic hypothesis, of course, necessitated more extensive cross-transmission studies involving a multitude of wild hosts to determine if, indeed, a wild species could be responsible for the epidemics.

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SECTION 2

CONCLUSIONS

Giardia cysts obtained from human, dog, cat and beaver are not host specific. *Giardia* from human sources will readily infect dogs, cats and beaver; *Giardia* from beaver sources will readily infect dogs; and *Giardia* from dog or cat sources readily cross-transmit between these species. Therefore it must be assumed that *Giardia* from dog, cat and beaver will readily infect humans.

SECTION 3

RECOMMENDATIONS

The results of this cross-transmission study have shown that the *Giardia* found in humans, dogs, cats and beaver are not host specific and will readily establish in the other species. All three species are important as potential sources of *Giardia* for epidemics of waterborne giardiasis, but the beaver probably plays the most important role because of its closer association with the water used by communities as their source of domestic supply. Therefore, a study of the host-parasite relationship between beaver and *Giardia*, together with a study of the factors predisposing an epidemic of waterborne giardiasis (water pH, hardness, temperature, etc.) is the next logical step necessary to understand waterborne giardiasis.

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SECTION 4

METHODS AND MATERIALS

Human feces containing Giardia cysts (Figure 1) were obtained from 3 sources in Fort Collins, Colorado: Colorado State University Student Health Center, Poudre Valley Memorial Hospital and the Northern Colorado Diagnostic Laboratory. All samples were submitted to these facilities for diagnostic purpose from clinically ill people and as far as known none were taking medication for Giardia. The source of the sample was not available. Beaver-source Giardia were obtained from naturally infected beaver on Beaver Creek near Hot Sulphur Springs, Colorado. Mule deer-source Giardia were obtained from a naturally infected mule deer fawn in a captive herd near Kremmling, Colorado. Giardia was obtained from naturally infected muskrats from the Poudre River near Bracewell, Colorado.

All fecal samples received from health facilities in Fort Collins were refrigerated and less than 12 hours old. Fecal samples collected from wild animals were placed in plastic bags and placed in a styrofoam chest containing ice. All fecal samples were examined within 24 hours by the $ZnSO_4$ centrifugation method (13:page 106) for the presence of Giardia cysts. If cysts were present and not distorted or the cyst contents not pulled away from the cyst wall, the cysts were considered viable. The entire fecal sample was then suspended in distilled water, thoroughly mixed and strained through a double thickness of cheese cloth. The container was rinsed with distilled water and the fluid poured through the cheese cloth. The cheese cloth and debris filtered out was then discarded. The filtrate was poured into 50 ml round bottom test tubes and centrifuged at 2300 rpm for 5 minutes. The supernatant was discarded, the sediment resuspended in distilled water and centrifuged again. This process was repeated until the supernatant was almost clear. The supernatant was once again discarded and the pellet resuspended in distilled water. This was allowed to stand for 1-5 minutes or centrifuged at 1,000 rpm for 10 seconds to sediment larger particles. The supernatant was then poured into a glass beaker and 5 0.1 ml samples taken and placed on a glass slide and covered with a cover slip for counting. All Giardia cysts were counted in each sample using a binocular microscope at 400X. The number of cysts per sample was averaged to obtain the concentration of cysts in the beaker. Fluid could then be removed or added to obtain a usable concentration of cysts in the water. The fluid containing cysts was used to inoculate animals within 2 hours following separation from the feces. If inoculation was delayed for more than 15 minutes after separation, the fluid was refrigerated until used. The fluid was thoroughly stirred before administration to an animal.

Animals were inoculated via stomach tube, orally by introducing fluid into the mouth or between the cheek and teeth or in water provided to animals

deprived of water for the previous 24 hours (used with raccoons). Ten thousand cysts in 1-5cc of water was used as the infective dose whenever possible. In some cases there were not enough cysts in the sample to give 10,000 cysts to each animal and as few as 2,000 cysts were given. In-group controls were separated from the exposed animals at the time of inoculation, given an equal volume of distilled water and held in a separate cage for 24 hours to prevent exposure due to regurgitation of fluid by exposed animals or from control animals licking the faces of exposed animals. The in-group control provided a means of monitoring exposure of animals to other sources of Giardia (such as city water which may be contaminated), an index of transmission from exposed animals after they start shedding cysts, as well as an indication of infected animals shedding cysts which were not detected by fecal examination. In most cases control animals were kept in identical cages or facilities and treated identically as infected animals. Control animals were not always available for beaver, raccoon, black bear or large wild ungulates.

Fecal samples from each animal, including controls, were examined daily by the $ZnSO_4$ centrifugation method for a minimum of 14 days prior to infection and many were monitored for nearly 2 months before a Giardia source was obtained. Fecal samples from each infected animal, in-group controls and control animals were examined daily for a minimum of 21 days. Animals were monitored to determine the prepatent and patent period.

Laboratory rats and mice, hamsters, guinea pigs, gerbils and domestic rabbits were obtained from the Experimental Animal Service at Colorado State University or local pet shops. Beaver, raccoon, deer mice, white-tailed deer, wapiti, pronghorn, bighorn X mouflon sheep and black bear were obtained from the Colorado Division of Wildlife or were captive animals held at the Colorado State University Foothills Campus. Large domestic animals (sheep and cattle) were obtained and held in pens at the Foothills Campus. Specific Pathogen Free (SPF) beagle puppies were obtained from the Collaborative Radiological Health Laboratory (CHRL) on the Foothills Campus. These dogs are used primarily for a nuclear exposure study. For more detail the reader is directed to the CHRL for more information. All animals were kept in pens at the Foothills campus (large ungulates) or in appropriate cages in animal rooms on the main campus, fed dry commercial feed and/or alfalfa hay. Fort Collins city water was provided as needed. Fort Collins water is both filtered and chlorinated, and is regularly monitored for Giardia.

When feasible, animals were killed and wet impression smears and smears from intestinal scrapings were made from various levels of the intestine. Wet smears were stained with Lugol's Iodine and examined immediately. A fecal sample was taken from the lower colon at necropsy and examined by the $ZnSO_4$ method.

SECTION 5

RESULTS

Animals exposed to Giardia cysts from clinically ill humans produced data which varied both within and among experimental groups (Tables 1 and 2). Hamsters (Mesocricetus auratus), domestic rabbits (Oryctolagus cuniculus), laboratory mice (Mus musculus), wapiti (Cervus canadensis), mule deer (Odocoileus hemionus), white-tailed deer (O. virginianus), black bear (Ursus americanus), domestic sheep (Ovis aries), and domestic cattle (Bos taurus) were not successfully infected with human source Giardia. An infected or positive animal is one in which a Giardia cyst or cysts were found in 1 or more fecal samples collected from the animal at least 36 hours after the animal was exposed to Giardia. Animals which did become infected were laboratory rats (Rattus norvegicus), gerbils (Gerbillus gerbillus), guinea pig (Cavia porcellus), beaver (Castor canadensis), dog (Canis familiaris), raccoon (Procyon lotor), bighorn X mouflon sheep (Ovis canadensis X O. musimon) and pronghorn (Antilocapra americana). Giardia cysts recovered from pronghorn and naturally infected mule deer were often distorted and approximately half the diameter of non-deformed cysts (Figures 1 and 2). Deformed cysts were seen when the feces of the animal was formed and dry. When feces from these animals was diarrhetic, cysts were plump and internal structures filled the cyst. Cysts from all animals, with the exception of some of these from rats, ranged in size from 9.5 to 11.0 μm x 8.0 to 9.5 μm. After exposed animals became patent the in-group control often started shedding cysts 8 - 20 days after exposed animals became patent. This indicates transmission of Giardia from exposed animals to the in-group control. None of the control groups, held in the same facilities as the exposed animals and the in-group control, became positive for Giardia.

The first group of rats (4 exposed and 1 in-group control) exposed to human source Giardia was kept in a single cage and composite fecals examined. At this time sufficient equipment was not available to permit obtaining fecals from each animal. All other experiments were performed so that each animal could be placed in a cage on wire until a fecal was obtained. Giardia cysts were recovered from the composite fecals from the first group on days 22, 25 and 40 post exposure (PE) (Table 1) and were of 2 sizes, one 5μm long, the other 10μm long, yet identical in all other aspects. One rat exposed to human Giardia in a second experiment shed cysts for 1 day, 34 days PE. All of the cysts were 10μm long x 8 μm wide. All other animals in this group, including the in-group control, remained negative for 40 days PE. All of the rats were killed at 40 days PE and trophozoites were not found in intestinal smears, scrapings or by $ZnSO_4$ centrifugation. Feces from the infected rat were fed to a beagle puppy which started shedding Giardia cysts 8 days PE. Control puppies remained negative.

A total of 4 groups of gerbils (each group consisting of 4 exposed and 1 in-group control) were exposed to human source Giardia (Table 1). Three of the 4 exposed animals were cyst positive between 13 and 18 days PE. These animals produced cysts in varying numbers and cysts were not consistently found in each sample. By 33 days PE, the in-group control was positive for Giardia. The remaining exposed animal remained negative through 45 days PE when the experiment was terminated. Of the next 2 groups (4 exposed and 1 in-group control) 1 exposed animal from each group shed Giardia cysts for 1 day 8 days PE. All other animals remained negative. The fourth group (4 exposed plus 1 in-group control) remained negative for 42 days when the experiment was terminated. Neither trophozoites nor cysts were recovered from any of the animals at necropsy.

One group of guinea pigs (4 exposed and 1 in-group control) was exposed to human source Giardia. One exposed animal became positive for Giardia 2 days PE and remained positive for 31 days. The remaining 4 animals were negative for 58 days PE when the experiment was terminated.

Three beaver were maintained in captivity for 40 days and checked daily for Giardia. All remained negative for 40 days when 2 were exposed to 10,000 human source Giardia cysts each (Table 1). All 3 remained negative for 38 days. As additional beaver were not available, the control animal and 1 previously exposed beaver were exposed to 10,000 Giardia cysts from a different human source. The other previously exposed beaver served as a control animal. Both of the exposed beaver started shedding Giardia cysts 25 days PE and shed cysts daily for 22 days. They then shed cysts intermittently with periods up to 7 or more days of no detectible Giardia cysts in the feces. The control remained negative during this period. Apparently the Giardia cysts from the first source were not infective while the Giardia from the second source were infective.

Three groups of 8-12 week old SPF beagle puppies were exposed to human source Giardia. In the first group of dogs both exposed dogs started shedding Giardia in the feces 6 days PE and the in-group control housed with the exposed dogs started shedding cysts 7 days later (13 days after the 2 dogs were exposed). The exposed dogs in the second group became positive 8 days PE with the in-group control shedding Giardia cysts 7 days later (15 days after the 2 other dogs were exposed). Two puppies (group 3) were exposed to a human source Giardia which had been shipped from Cincinnati, Ohio. Due to delays by the airline the fecal sample suspended in water was held for 4 days at room temperature and when examined the cysts were deformed and did not stain well with Lugol's Iodine. None of the dogs exposed to these cysts became infected.

A total of 10 raccoons were exposed to human source Giardia from 4 different sources (Table 1). One young raccoon was positive for 1 day, 8 days PE, with 3 cysts being found. One black bear cub about 5 months old, was exposed to a minimum of 10,000 Giardia cysts from a human source but remained negative.

Four bighorn X mouflon sheep were exposed to 10,000 human source Giardia cysts (Table 1). Two of the 4, and the in-group control animal, remained

negative while the other 2 animals, #29 and #44, shed Giardia cysts for 4 days starting 9 days PE. Both animals had diarrhea while shedding cysts. Giardia cysts were isolated from both infected sheep and 10,000 cysts from each animal given to a SPF beagle pup. The pup given Giardia cysts from #29 became positive 10 days PE while the pup given cysts from sheep #44 remained negative. The pup which was given cysts from sheep #44 was later successfully infected with human source Giardia.

One other ungulate was successfully infected with human source Giardia, a pronghorn antelope fawn approximately 3 months old. This animal shed deformed cysts (Figure 2) for 3 days, 16-18 days PE. The dry pelleted feces of this animal is the most likely cause of the deformed cysts. Diarrhea or soft feces was not observed in this animal.

All attempts to infect wapiti, mule deer, white-tailed deer and domestic sheep and cattle failed (Table 1). However, naturally infected mule deer (Figure 3) and cattle have been found (13). An attempt to infect muskrats must also be considered a failure as all animals, including the control, were found positive 18 hours after being exposed to human source Giardia.

Results from a series of experiments exposing kittens and puppies to human source Giardia and puppies to kitten source Giardia are shown in Table 2. Eighteen of 20 kittens and 4 of 4 puppies exposed to human source Giardia became infected with prepatent periods ranging from 6-27 days in kittens and 6-10 days in puppies. Feces were collected from 1 of the kittens (Group B, #2), which had been infected with human source Giardia, and cysts (Figure 4) were given to a group of kittens and a group of puppies. Three of the 4 kittens became positive as did 3 of 4 puppies. All control animals were held in identical cages in the same facility and all remained negative throughout these experiments (Tables 1 and 2).

TABLE 1. RESULTS OF EXPERIMENTAL INOCULATION OF EXPERIMENTAL ANIMALS WITH GIARDIA FROM HUMAN SOURCES

Experimental animal*	Results
Hamster (Mesocricetus auratus) (4+1)	All animals including controls remained negative for 34 days Post Exposure (PE).
Domestic rabbit (Oryctolagus cuniculus) (4+1)	All animals including controls remained negative for 28 days PE.
Laboratory mice (Mus musculus) 4 groups of (4+1)	All animals, including controls, remained negative for 40 days PE.
Rats (Rattus norvegicus) (4+1)	Cysts found in feces on days 22, 25 and 40. Cysts were of 2 sizes and

(cont.)

TABLE 1. (continued)

	animal(s) which were shedding cysts could not be identified.
(4+1)	One exposed rat shed cysts for 1 day 34 days PE. Cysts from this rat did produce infection in a SPF beagle puppy. The other 3 exposed rats and controls remained negative.
Gerbils (Gerbillus gerbillus) (4+1)	#1 - exposed - positive days 22, 26-29, 32-33 PE #2 - exposed - positive days 13, 19-22, 26-28 PE #3 - in-group control - became positive days 33-35 #4 - exposed - positive days 18-21, 26 PE #5 - exposed - remained negative 45 days PE
(4+1)	Exposed and control animals remained negative for 42 days PE
Guinea Pig (Cavia porcellus) (4+1)	One exposed animal started shedding cysts on day 21 PE and shed cysts for 31 days. The other 3 exposed animals and the control animal remained negative.
Beaver (Castor canadensis) (2+1)	All animals, exposed and control remained negative.
(2+1)	Both exposed animals positive 25 days PE and continued to shed cysts for 22 days. The control animal remained negative.
Muskrat (Ondatra zibethica) (2+1)	All animals, exposed and controls, were positive 18 hrs. PE. Animals most likely were already infected.
Dog (Canis familiaris) (2+1)	All exposed animals became positive 6 days PE and shed cysts for 14 days and were then treated. In-group control became positive day 13; other control animals remained negative.
(2+1)	All exposed animals became positive 8 days PE and shed cysts for 14 days and were then treated. The in-group control became positive day 15; other control animals remained negative.
(2+2) (cont.)	All animals, exposed and controls, remained negative. Cysts were shipped

TABLE 1. (continued)

	from Cincinnati, Ohio, and arrived in poor condition.
Raccoon (*Procyon lotor*) (3+1)	All animals, exposed and controls, remained negative 28 days PE.
(3+1)	All animals, exposed and controls, remained negative 43 days PE.
(3+1)	All animals, exposed and controls, remained negative 34 days PE.
(1)	Positive 8 days PE for 1 day.
Black Bear (*Ursus americana*) (1)	Animal remained negative for 28 days PE.
Bighorn X mouflon sheep (*Ovis canadensis X O. musimon*) (4+1)	# 1 - Negative # 2 - Negative #12 - Control - Negative #29 - Positive 9 days post-exposure for 4 days #44 - Positive 9 days post-exposure for 4 days
Wapiti (*Cervus canadensis*) (2+1)	All animals remained negative 30 days PE.
(2+1)	All animals remained negative 28 days PE.
Mule deer (*Odocoileus hemionus*) (2+2)	All animals remained negative 28 days PE.
(4+2)	All animals remained negative 30 days PE.
(3+6)	All animals remained negative 22 days PE.
White-tailed deer (*O. virginianus*) (1+2)	All animals remained negative 30 days PE.
(1+6)	All animals remained negative 22 days PE.
Pronghorn (*Antilocapra americana*) (1+1)	Exposed animal was positive days 16-18 PE. Control remained negative.

(continued)

Domestic sheep (Ovis aries) (3+1)	TABLE 1. (cont.)	All remained negative for 45 days PE.
Domestic cattle (Bos taurus) (4+1)		All animals remained negative for 45 days PE.

*Number of exposed animals + number of in-group controls)
Other controls were kept in the same facility.

TABLE 2. RESULTS OF EXPERIMENTAL INFECTIONS USING HUMAN SOURCE GIARDIA INFECTING DOGS AND CATS AND BETWEEN DOGS AND KITTENS.

Giardia Source	Animal Infected (#Positive/Exposed)	Mean Prepatent Period (Range)
Human Source A	Kitten (2/4)	18.0 days (17,19)
Human Source B	Kitten (4/4)	18.5 days (11-27)
Human Source C	Kitten (4/4)	8.0 days (7-10)
Human Source D	Kitten (4/4)	7.0 days (6- 8)
Human Source E	Kitten (4/4)	10.0 days (6-16)
Human Source C	Puppy (4/4)	8.5 days (6-10)
Kitten (B2)	Kitten (3/4)	14.0 days (10-17)
Kitten (B2)	Puppy (3/4)	19.7 days (12-29)

The mean prepatent period of human source A and B is significantly different ($P < 0.005$) from the mean prepatent period of human source C, D, and E.

Giardia cysts recovered from feces of naturally infected free-ranging beaver were given to mice, rats, hamsters and guinea pigs, all of which remained negative (Table 3). Puppies given Giardia cysts from the same source became positive 8 days PE and the in-group control puppy was positive 9 days later (17 days after the exposed dogs were inoculated).

TABLE 3. RESULTS OF EXPERIMENTAL INFECTIONS USING BEAVER (CASTOR CANADENSIS SOURCE GIARDIA.
(continued)

Experimental Animal*	TABLE 3. (cont.) Results
Mice (Mus musculus) 2 groups (4+1)	All negative
Rats (Rattus norvegicus) 2 groups (4+1)	All negative
Hamsters (Mesocricetus auratus) (4+1)	Negative - 30 days
Guinea pig (Cavia porcellus) (4+1)	Negative - 30 days
Dog (Canis familiaris) (4+1)	All 4 exposed animals positive 8 days after exposure - control positive 9 days later

*Number of animals infected followed by the number of in-group controls.

Two groups of dogs were exposed to Giardia cysts isolated from muskrat feces (Table 4). At 4 days PE all 4 dogs (2 exposed and 2 controls) were shedding Giardia cysts. Most likely these puppies had been exposed to Giardia before the experiment was started. A second group of puppies were exposed to muskrat source Giardia but all remained negative for the 63 days they were monitored. Two puppies from this second group were later exposed to a human source Giardia, but neither became positive.

TABLE 4. RESULTS OF EXPERIMENTAL INFECTION USING MUSKRAT (ONDATRA ZIBETHICA) SOURCE GIARDIA CYSTS.

Experimental Animal*	Results
Puppies (Canis familiaris) (2+2)	Exposed on 8 July 1979 all positive on 12 July - controls were in separate cage; 14 July - 3 of 4 pups negative 15 July - 4 of 4 pups negative
Puppies (2+2) (cont.)	All negative 63 days post-exposure (two of these pups were later exposed to human source Giardia but remained negative)

TABLE 4. (continued)

*Number of animals infected followed by the number of in-group controls.

Giardia cysts from a naturally infected mule deer were given to 2 SPF beagle puppies (Table 5). The inoculated and controls remained negative for 28 days after which the experiment was terminated.

TABLE 5. RESULTS OF EXPERIMENTAL INFECTIONS USING MULE DEER (ODOCOILEUS HEMIONUS) SOURCE GIARDIA.

Experimental Animal*	Results
Dog (Canis familiaris) (2+5)	Negative 28 days

*Number of exposed animals followed by number of in-group controls.

A group of 8 puppies was obtained from the Experimental Animal Service and when checked, 7 of the 8 were positive for Giardia. This group was used to determine the effectiveness of Flagyl® as an anti-Giardia medication and if it was possible to re-infect dogs apparently cured of a Giardia infection. Six of the 8 puppies were given Flagyl® at the rate of 30 mg/-lb/day for 5 days. The 6 treated puppies were negative for Giardia by the end of the third day of treatment. Seven days after the last day of treatment, 5 of the 6 treated puppies were given 10,000 cysts each from a human source. None of the puppies shed Giardia cysts for 40 days PE. The untreated puppy continued to shed cysts, and cysts were not seen in the puppy which was negative when the puppies were obtained. At 40 days PE all 8 dogs were euthanized and intestinal scrapings made and examined for Giardia trophozoites. Three of the 5 puppies which were exposed to human Giardia had trophozoites but none were shedding cysts (Table 6). The 1 puppy which was treated but not exposed to Giardia did not have any trophozoites. This experiment would have provided more data if 2 or 3 of the puppies had been euthanized and examined for trophozoites after being treated with Flaygyl®.

TABLE 6. RESULTS OF FLAGYL® DRUG TRIAL AND REINFECTION OF PUPPIES WITH HUMAN SOURCE GIARDIA.

Puppy Number	Results
1.	Never positive for Giardia - litter mates were positive for Giardia - trophozoites were not found at necropsy

(cont.)

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TABLE 6. (continued)

2.	Treated - reinfected - not positive at necropsy
3.	Treated - reinfected - Trophozoites found in duodenum, ileum and jejunum
4.	Treated - not reinfected - Negative at necropsy
5.	Treated - reinfected - Trophozoites found in jejunum and ileum
6.	Treated - reinfected - Negative at necropsy
7.	Treated - reinfected - Trophozoites in duodenum
8.	Not treated - Trophozoites in duodenum, jejunum, ileum

As this study progressed, it became increasingly difficult to find puppies and kittens which were Giardia free. In order to obtain clean animals, pregnant dogs and cats were obtained from the Experimental Animal Service and held in clean rooms until they gave birth and offspring were weaned. Of the first 3 pregnant dogs handled in this method it was determined that all 3 female dogs started shedding Giardia cysts 2-4 weeks after parturition and all offspring would be positive within 7 days after the female started shedding cysts. Part of the criteria for choosing a pregnant dog was that they were Giardia free for 7 days prior to acceptance. Fecals were collected and examined daily and none of the dogs shed cysts until 2-4 weeks after parturition. The solution to this problem was to treat the adult animal with Flagyl® at 30 mg/lb/day for 5 days. Dogs and cats treated in this manner produced Giardia-free offspring and the adult did not produce Giardia cysts after parturition.

SECTION 6

DISCUSSION

The first and most important assumption of this study is that if Giardia from man will infect an animal, then Giardia from that animal will also infect man. The scope of this study does not cover the use of human subjects and therefore the hypothesis that animal Giardia will infect humans remains untested.

Giardia has long been a misunderstood parasite, yet this study does little more than document its transmissibility between species and raises many additional questions. We now know dogs, cats and beaver are reservoirs of Giardia and may be very important in the epidemiology of Giardia (13). With man's close association with his pets, humans may well be in a situation of high risk unless they have their pets checked for Giardia. Beaver are undoubtedly an important factor in sylvatic Giardia, but the source of the beaver infections is most likely man and his lack of wilderness sanitation and release of incompletely treated sewage into water supplies (13).

The problems encountered in all experiments were: (1) the viability of the Giardia cysts was unknown; and (2) the susceptibility of the experimental animal to infection was unknown. These points were brought out when puppies were exposed to human Giardia and did not become infected, yet when exposed to Giardia from another human source infection did occur. In another instance beaver exposed to human source Giardia did not become infected but when given Giardia from another human source, infection did occur. Variable results within groups of animals shows that some animals are not susceptible to the parasite even though litter mates were. One must remember that the viability of the cysts and the susceptibility of the experimental animals is unknown when interpreting the data.

Most human fecal samples obtained consisted of very small amounts of material which prevented exposure of several groups and/or species of animals. With an adequate amount of material, many more cysts can be isolated and several groups and/or species known to be susceptible can be exposed. This approach should answer the question of infectivity of the Giardia cysts. The lack of adequate numbers of cysts was often a problem during this study. Anyone conducting a study like this one would be advised to find a person who is an asymptomatic carrier of Giardia, determine the infectivity of the cysts by infecting puppies and using one person as a source of cysts throughout the study.

Dogs, cats and very possibly other animals can have latent infections of Giardia and not shed any cysts. This was best documented in 4 pregnant dogs,

1 of which had been monitored for 2 years with no Giardia being found in the feces, yet all started shedding Giardia cysts 2-4 weeks after whelping. Other animals held in the same facility, provided with the same food and water, remained negative. This latent infection phenomenon was also shown during drug trials. These animals apparently develop enough response to prevent cyst formation after being infected. This may also be the natural host-parasite response: one of overt disease (diarrhea and passage of cysts) followed by a depressed activity by the parasite and no cysts being passed. Once a stress or hormonal change occurs at parturition, the parasite resumes peak activity at the time the young will be exposed resulting in transmission of the parasite. This topic should be pursued by more research.

As the Giardia cysts recovered from all infected animals were close to the same size (except for that recovered from the first group of rats) and morphologically no difference was detected, only one species of Giardia, G. lamblia, was being worked with. We believe the rats were infected with G. muris, which is smaller than G. lamblia of other animals, before being exposed to human Giardia. It is very possible that the parasite adapts to a species. During cross-transmission experiments it was noted that the prepatent period was less if the species of animals was the same. If Giardia was given to a group of animals from a source X, the prepatent period may be 10 days. Yet if cysts from this now-infected group of animals is given to another group of the same species, the prepatent period may be shorter by several days. Thus it is likely that the Giardia infecting a species physiologically adapts to that species resulting in a shorter prepatent period in other members of the same species.

It was noticed in many animals that when they first started to shed cysts or when the number of cysts were decreasing as the animal stopped shedding cysts, the cysts did not stain well with Lugol's iodine and were often transparent. These cysts are extremely difficult to see and false negatives may have been reported. The muskrats exposed to human source Giardia which were found to be positive 18 hours PE shed cysts of this type for more than a week after they were first found. It is possible that this type of cyst is shed in small numbers during the latent infection period and the cysts simply overlooked.

Puppies appear to be an excellent experimental model for future Giardia research. Workers are recommended to infect at least one group of dogs along with any other species to determine viability of the cysts. From work completed we believe any Giardia which will infect puppies less than 6 months of age will also be infective for humans, although exceptions may occur. The major problem will be obtaining puppies which have not been exposed to Giardia. The best way will be to establish a dog colony, which will be very expensive, and treat all female dogs before they whelp.

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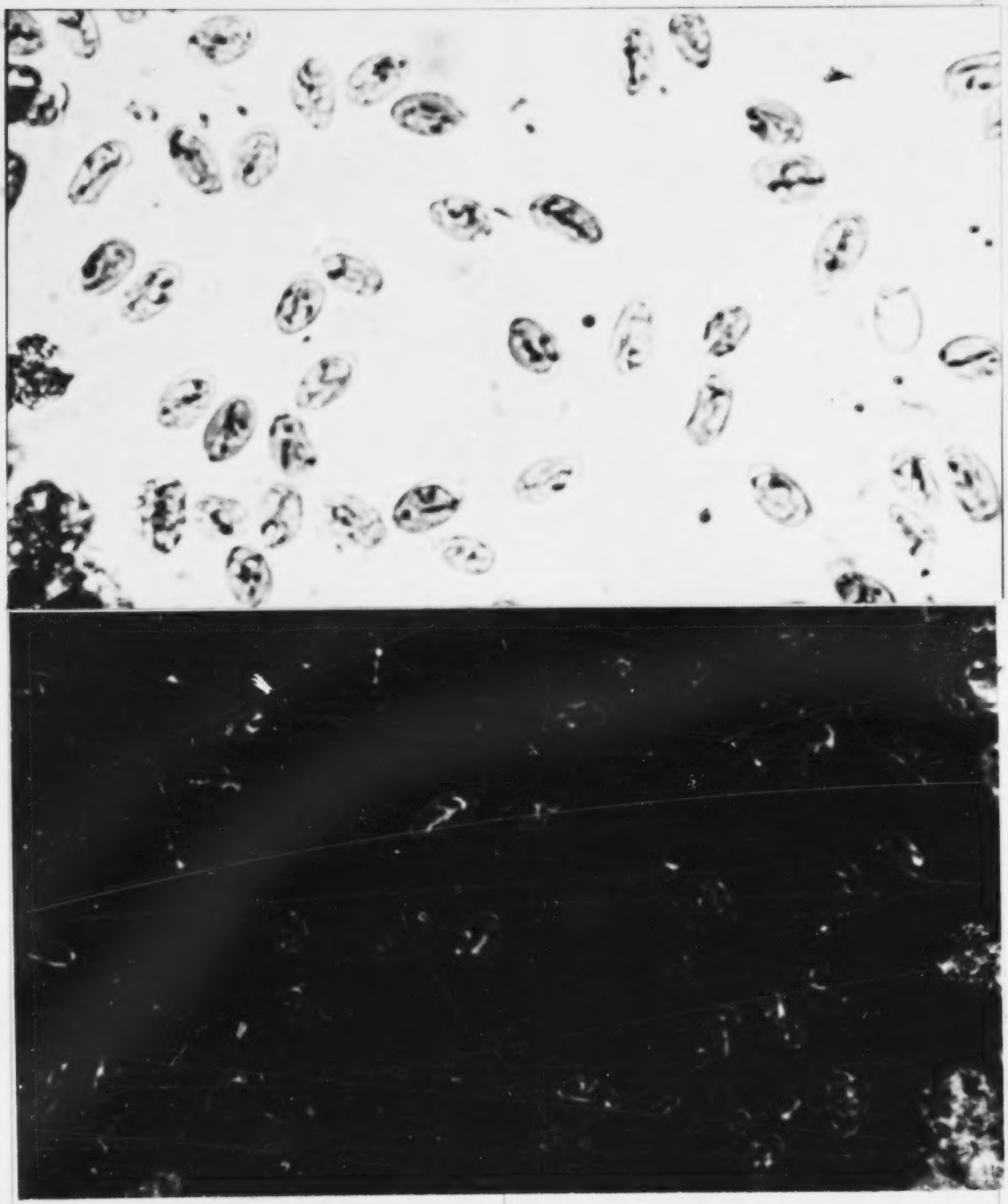

FIGURE 1. Giardia cysts from a human source. The cyst contents fill the cyst and stain well. 1400X.

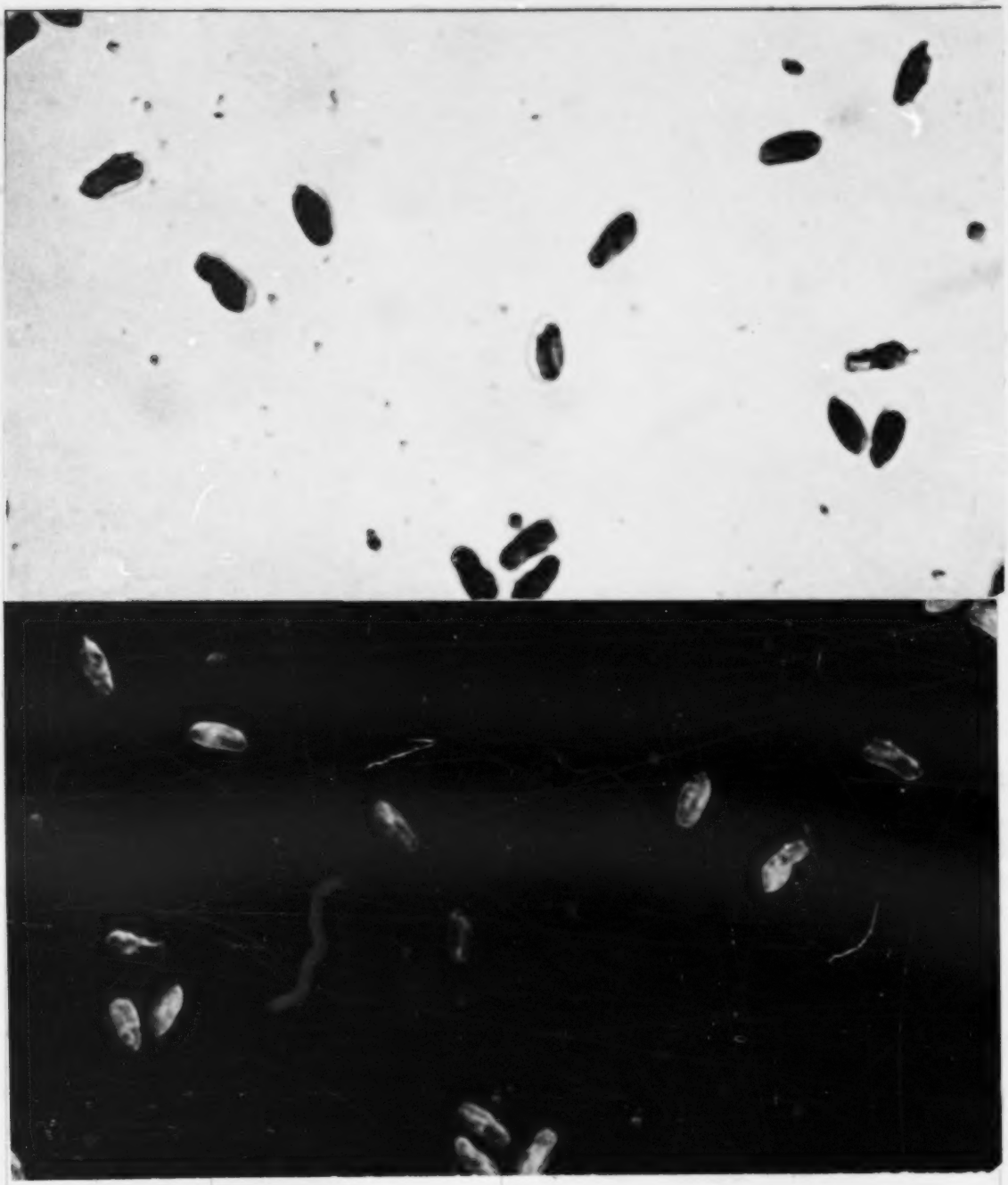

FIGURE 2. Giardia cysts from a pronghorn (Antilocapra americana). Note how the cyst contents are shrunken and pulled away from the cyst wall and the dark staining of the contents. 1300X.

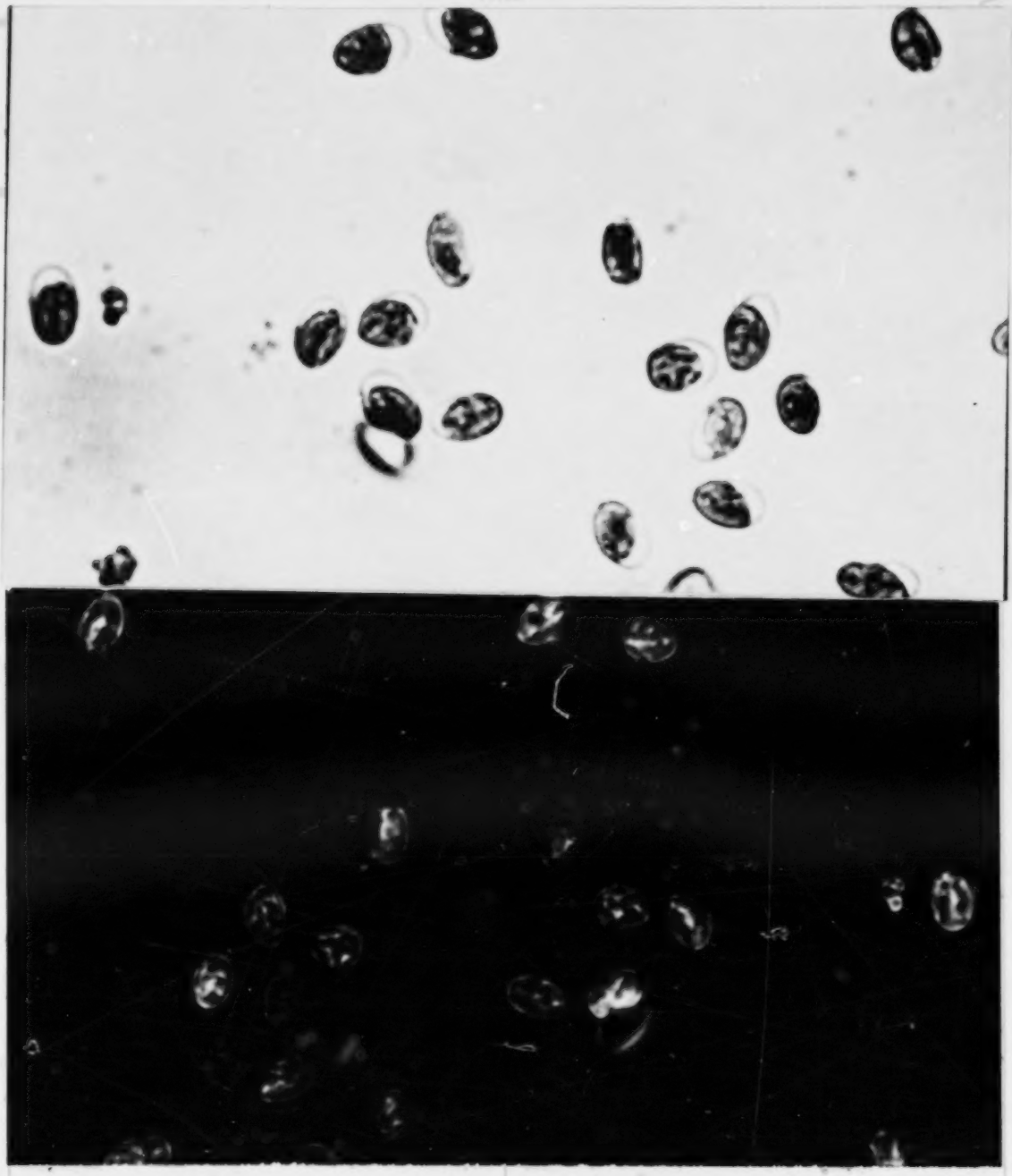

FIGURE 3. Giardia cysts from a mule deer (Odocoileus hemionus). Most of the cyst contents are pulled away from the cyst wall, yet internal structures are easily seen. compare these to thise in Figures 1, 2, and 4. 1300X.

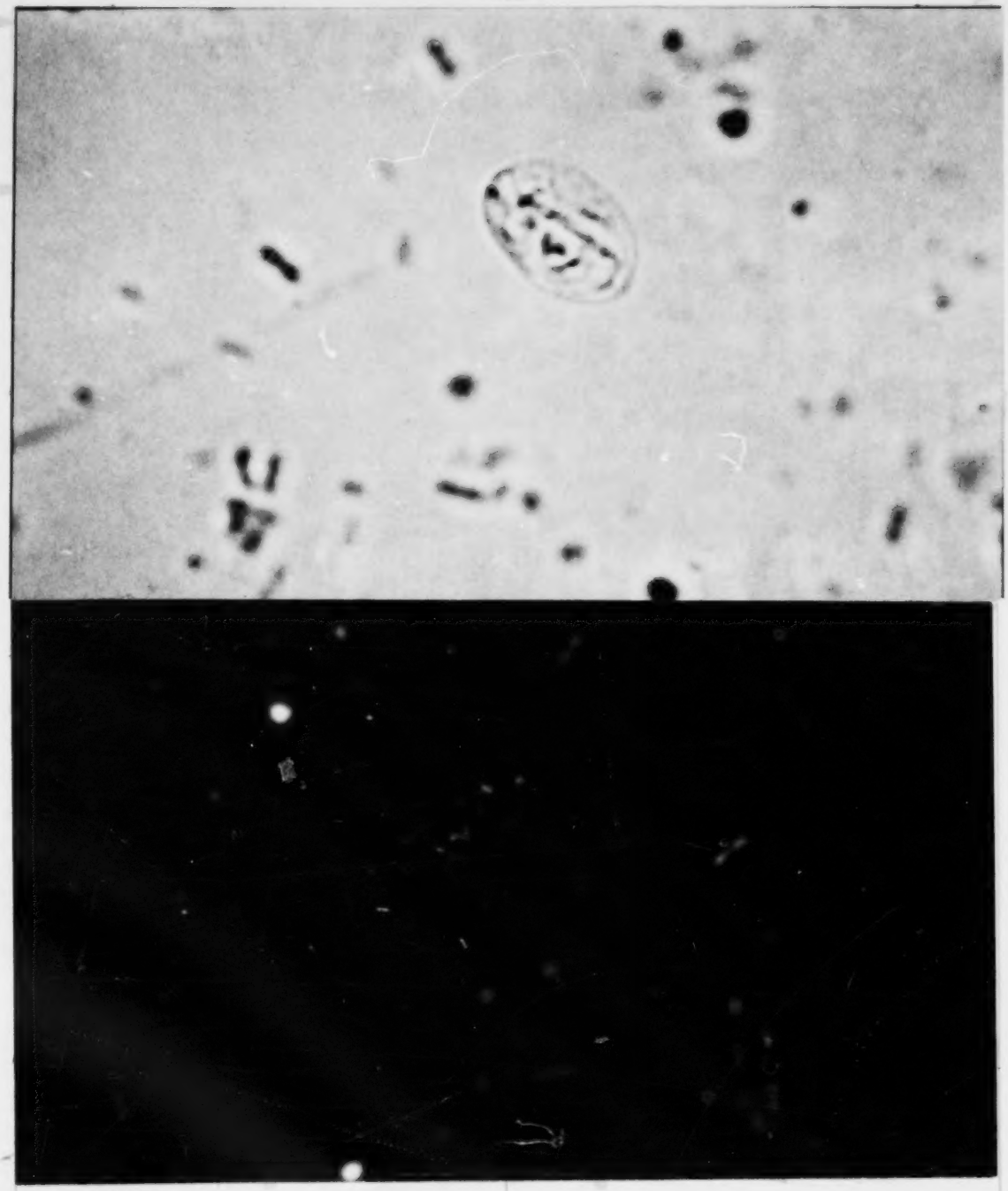

FIGURE 4. Giardia cyst from a domestic cat. Cyst is morphologically identical to cysts from humans and other animals. 3000X.

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